QnAs with Alexander Levitzki

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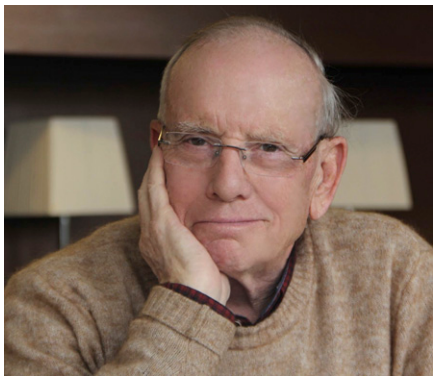
Alexander Levitzki has made significant contributions to the fields of enzymology, signal transduction, and cancer research. Early in his career, Levitzki studied allosteric enzymes, followed by key discoveries about the signal transduction of G protein-coupled receptors, particularly the coupling of beta-adrenergic receptors with G proteins, which are central to cellular signaling pathways. Levitzki also developed the first inhibitors of oncogenic protein tyrosine kinase enzymes, and this pioneering work laid the foundation for the development of a range of anticancer drugs. He has recently been working on targeting tumors with polyinosine/polycytosine acid (PolyIC) in hopes of marshaling the immune system to attack tumors. A professor of biochemistry at The Hebrew University of Jerusalem, Levitzki was elected as a foreign associate of the National Academy of Sciences in 2017. In his Inaugural Article, Levitzki describes the development of targeted cancer therapies (1).

PNAS: How did you transition from your early work in enzymology to anticancer therapies?

Levitzki: For many years, I worked on allosteric regulation of proteins and binding of proteins to different ligands, and the dynamic behavior of these proteins in the presence of these ligands. This kind of enzymology was a good base when I started to look for inhibitors of tyrosine kinases that are key to cancer biology. Since the ATP-binding domain is highly conserved, the kinase domains are very similar among protein kinases. Since there are a lot of enzymes using ATP as a substrate, it was not believed possible to generate a small molecule that would be selective enough to inhibit tyrosine kinases, let alone specific tyrosine kinases, so there was quite a bit of skepticism around that idea. On these grounds, my NIH grant detailing our approach to generate specific tyrosine phosphorylation inhibitors (tyrphostins) was rejected in 1986. We nevertheless went ahead and even published the data of the grant proposal in *Science* in 1988 (2). Our confidence stemmed from our understanding of enzyme specificity, which told us that minute differences in the active site are sufficient to generate selectivity. For example, the degree of homology between trypsin, chymotrypsin, and elastase is very high, yet the subtle differences among the active sites generate the selectivity of these enzymes.



Alexander Levitzki. Image courtesy of Smadar Bergman (Israel Institute for Advanced Studies, Jerusalem).

PNAS: How did your successful development of the first tyrosine kinase inhibitors influence the field of cancer therapeutics?

Levitzki: Our work in the field of kinase inhibitors was really pioneering. Nowadays, there are dozens of anticancer kinase inhibitors in the clinic and in clinical development, and all of them emanate from the first concept that we developed. The first kinase inhibitor anticancer drug, Gleevec, was made by following up on the work we did on tyrphostins aimed at Bcr-Abl in 1992–1993.

PNAS: What have been some of the challenges of using targeted therapies against cancer?

Levitzki: Tyrosine kinase inhibitors are an important advance, but they may not be sufficient by themselves. Cancer is a complex disease, and every individual cancer is different. Cancer is like an organ that is composed of tumor cells and the tumor microenvironment, which cooperate together. A single drug will never be good enough to treat it, and one would have to use mixtures of drugs, either different drugs together or consecutive treatment with different drugs for the same patient, over a period of time.

PNAS: What advances have you made in developing multitargeted tyrosine kinase inhibitors?

Levitzki: We have developed compounds called NT157 and NT219 that target not only IGF1 receptor kinase signaling but also STAT3, and both of these elements are key to many tumors. NT157 and NT219 are multitargeted compounds that target not only the

This QnAs is with a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 11579.
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tumor but also the tumor microenvironment in which IGF1 signaling and STAT3 signaling are key elements. NT157 and NT219 therefore deal with the heterogeneity of the signaling network of the tumor and its microenvironment. Together with Michael Karin, we published a paper (3) showing that NT157 has very profound effects on an animal model of colon cancer because of the fact that it is a multitargeted compound. It's not in the clinic yet, but it is in clinical development by TyrNovo of Tel Aviv. NT157/NT219 [could] become useful for many tumors as an add-on drug that improves current therapies used for different cancers.

PNAS: How did you become interested in using the synthetic long chain double-stranded RNA PolyIC as a cancer therapy?

Levitzki: PolyIC has been used for many years as an immunoadjuvant. Back in the 1980s, people attempted to use it as an anticancer agent by systemic injection. It has been known for a long time that PolyIC is a wake-up signal for the immune system. However, the systemic application of PolyIC is too toxic because it creates a cytokine storm in the patient, which blocked further clinical development. We argued that PolyIC is likely to become effective when it is targeted to the tumor, and this would allow us to resurrect it as a therapeutic modality. For the past 13 years, we have been working toward this end.

We have been developing the methodology to target PolyIC to the EGF receptor overexpressed in many tumors; to Her2 overexpressed in breast cancer; and to PSMA, which is overexpressed in metastatic prostate cancer. Targeted PolyIC is not yet in the clinic, but it's in advanced preclinical studies by TargImmune Therapeutics of Basel.

PolyIC invokes a few anticancer signaling mechanisms at the same time, so the probability of the cancer developing resistance to the therapy is very small. The immune system is really the key to tracking down cancer cells wherever they are, and that's why I think that recruiting the immune system together with the targeted therapies that we've been using can be a very effective combination.

PNAS: What do you see as the future of anticancer therapies?

Levitzki: The not-too-distant future is going to involve developing smart combinations based on informational analysis of the tumors in individual patients to develop a patient-oriented drug mixture or mixtures. It is going to involve the further development of currently available therapies, and there will be more types and combinations of therapies that will be developed for different cancers. I think immune therapies will continue to develop, probably by developing more antibodies and CAR-T therapies as well as, hopefully, more targeted PolyIC therapies. I believe cancer therapies will move toward patient-oriented protocols.

1 Levitzki A, Klein S (2019) My journey from tyrosine phosphorylation inhibitors to targeted immune therapy as strategies to combat cancer. *Proc Natl Acad Sci USA* 116:11579–11586.

2 Yaish P, Gazit A, Gilon C, Levitzki A (1988) Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. *Science* 242: 933–935.

3 Sanchez-Lopez E, et al. (2016) Targeting colorectal cancer via its microenvironment by inhibiting IGF-1 receptor-insulin receptor substrate and STAT3 signaling. *Oncogene* 35:2634–2644.